J. Pharm. Pharmacol. 1985, 37: 352–354
Communicated September 5, 1984

Monoamine oxidase-A selective inhibition in human hypothalamus and liver in-vitro by amiflamine and its metabolites

KEITH F. TIPTON‡, ANNE-MARIE O'CARROLL†, JOHN M. MCCRODDEN, CHRISTOPHER J. FOWLER*, *Department of Biochemistry Trinity College, Dublin 2, Ireland and *Research and Development Laboratories, Astra Läkemedel AB, S-151 85 Södertälje, Sweden.*

Amiflamine (FLA 336(+)) and its two metabolites, FLA 788(+) and FLA 668(+) were found to be competitive inhibitors of the activity of monoamine oxidase-A in homogenates of human hypothalamus and liver obtained at autopsy. K_i values, determined at pH 7·2, were 1·3, 0·3 and 22 μM (liver) and 0·8, 0·2 and 14 μM (hypothalamus) for amiflamine, FLA 788(+) and FLA 668(+), respectively. Monoamine oxidase-B activity was only weakly inhibited by the compounds.

The mitochondrial enzyme monoamine oxidase (MAO, EC 1.4.3.4) exists as two catalytically active forms, termed MAO-A and -B. In the human brain and liver, 5-hydroxytryptamine is deaminated essentially by MAO-A alone, whereas 2-phenylethylamine is deaminated predominantly by MAO-B (Hall et al 1969; Tipton et al 1973; O'Carroll et al 1983) although there is some crossover at high substrate assay concentrations (see Fowler & Tipton 1984). The α-methyl substituted monoamine amiflamine ((S)-(+)-4-dimethylamino-α,2-dimethylphenethylamine, FLA 336(+)) has been shown in-vitro, both in rat and in man, to be a reversible competitive MAO-A selective inhibitor (Ask et al 1982; Fowler & Oreland 1980). In-vivo experiments in the rat have shown that amiflamine is accumulated preferentially (via the active uptake mechanisms) within 5-HT neurons (Ask et al 1983) and does not potentiate the effects of orally administered tyramine until doses much higher than necessary for MAO-A inhibition are given (Lindbom et al 1983). In the rat, amiflamine is extensively demethylated to form desmethylamiflamine (FLA 788(+)), and it is this compound which exerts the MAO inhibitory actions in-vivo (Ask et al 1982). In man, however, FLA 788(+) is further metabolized to yield didesmethylamiflamine (FLA 668(+)) (Graffner et al 1983). Since little is known about the inhibitory effects of these two metabolites on MAO-A and -B in man in-vitro, it was decided to study their effects on human hypothalamus and liver obtained at autopsy.

Methods

Human liver and hypothalamus samples were obtained at autopsy. In no case did the death-autopsy delay exceed 16 h. A previous study has demonstrated that neither K_m nor V_{max} values for either form of human brain MAO are affected to any great degree by increasing death-autopsy delay (Fowler et al 1980). The samples were homogenized 1 : 5 (w/v) in 100 mM potassium phosphate buffer, pH 7·2, followed by sonication as decribed elsewhere (O'Carroll et al 1983). The homogenates were assayed for protein (Markwell et al 1978) and set to a protein concentration of approximately 10 mg ml^{-1} (hypothalamus) and 4 mg ml^{-1} (liver). These were then stored frozen at −20 °C until used for assay. MAO-A and -B activities were assayed at 37 °C by the method of Otsuka & Kobayashi (1964) with some minor modifications, as described elsewhere (O'Carroll et al 1983) in 50 mM phosphate buffer, pH 7·2, with 100 μM [^{14}C]5-hydroxytryptamine and 10 μM [^{14}C]2-phenylethylamine (obtained from Amersham International plc, Amersham, UK) as substrates for MAO-A and -B, respectively. Time-courses were determined to ensure that all velocity measurements were made in the period where product formation was linear with time. Amiflamine, FLA 788(+) and FLA 668(+) were synthesized by Dr L. Florvall, Astra Läkemedel AB, Södertälje, Sweden.

Results and discussion

In agreement with studies performed in the rat brain (Ask et al 1982; Fowler et al 1984), amiflamine, FLA 788(+) and FLA 668(+) were found to be MAO-A selective inhibitors. The IC50 values for the inhibition of the deamination of 100 μM 5-hydroxytryptamine by the three compounds were 0·78, 0·78 and 32 μM (hypothalamus) and 1·8, 0·63 and 13 μM (liver), respectively. The compounds were very weak inhibitors of MAO-B, with only 10, 5 and 2% (hypothalamus) and 12, 5 and 15% (liver) inhibition of the deamination of 10 μM 2-phenylethylamine being found for 100 μM amiflamine, FLA 788(+) and FLA 668(+), respectively (Data as mean, n = 3–6). The inhibition of MAO-A produced by all three compounds was found not to be dependent upon prior preincubation of the homogenate with the inhibitor, and to be fully reversible in nature as assessed by enzyme dilution techniques of the type described elsewhere (Tipton et al 1983).

Kinetic studies of the inhibition of the human hypothalamic and liver MAO-A activities towards 5-hydroxytryptamine by amiflamine, FLA 788(+) and FLA 668(+) indicated that the inhibition was in each case competitive. The K_i values obtained are given in Table 1 and the kinetic plots of the inhibition of

† Present address: Department of Long-term Care and Geriatrics, University of Umeå, S-901 85 Umeå, Sweden.
‡ Correspondence.

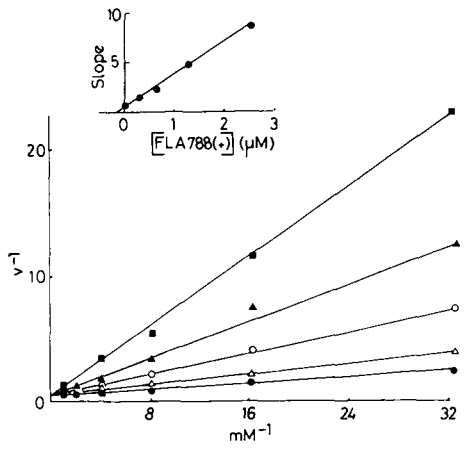

FIG. 1. Inhibition of human hypothalamic MAO-A by FLA 788(+). Ordinate: 1/Initial velocity (arbitrary units); abscissa: 1/5-hydroxytryptamine concentration in mM. Samples were assayed for activity, without preincubation, in the absence (●) or presence of 0·312 μM (△), 0·625 μM (○), 1·25 μM (▲) and 2·5 μM (■) FLA 788(+). The data represents the mean of three experiments. The secondary slope replot is shown as an inset.

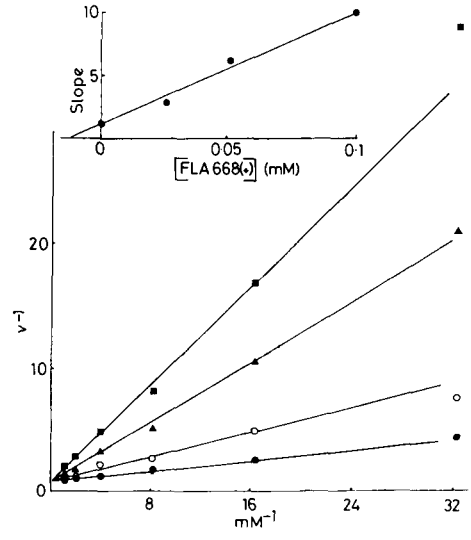

FIG. 2. Inhibition of human hypothalamic MAO-A by FLA 668(+). Ordinate and abscissa as for Fig. 1. Samples were assayed for activity, without a preincubation phase, in the absence (●) or presence of 25 μM (○), 50 μM (▲) and 100 μM (■) FLA 668(+). The data represents the mean of three experiments. The secondary slope replot is shown as an inset.

hypothalamic MAO-A by FLA 788(+) and FLA 668(+) are shown as examples in Figs 1 and 2. The differences in the values determined for the enzyme activities in liver and hypothalamus are not great and in view of the variability inherent in such determinations with different samples it would be inappropriate to regard them as being significant. From these data, it is apparent that FLA 788(+) is about 70 times more potent an inhibitor of MAO-A than FLA 668(+). This in turn would suggest that in man, despite the formation of both FLA 788(+) and FLA 668(+) after amiflamine administration (Graffner et al 1983), and despite different neuron selectivities of the two compounds (5-hydroxytryptaminergic for FLA 788(+), noradrenergic for FLA 668(+)) (see Ask et al 1983), the inhibition produced by amiflamine is most likely due to the pharmacological action of FLA 788(+) alone.

Table 1. Inhibitor constants for the competitive inhibition of human hypothalamus and liver MAO-A by amiflamine, FLA 788(+) and FLA 668(+). Data of the type shown in Figs 1 and 2, with three separate velocity determinations for each substrate-inhibitor concentration pair, were fitted to rectangular hyperbolae by unweighted non-linear least squares analysis. The apparent values of K_m/V(Slope) determined in this way were fitted to straight line functions of the inhibitor concentrations by linear regression analysis to determine the K_i values. The theory and computer program used have been given by Cleland (1979). The values are given as means ± s.e. determined from these fitting procedures.

Compound	K_i (μM) Liver	Brain
Amiflamine	1·3 ± 0·1	0·8 ± 0·08
FLA 788(+)	0·3 ± 0·05	0·2 ± 0·01
FLA 668(+)	22 ± 1·5	14 ± 3

The authors are indebted to Professor Jack Phillips, Dr Igdam Tobbia and the staff of St Laurence's Hospital, Dublin, for the supply of the autopsy material used in the study; and to the Medical Research Council of Ireland for financial support.

REFERENCES

Ask, A.-L., Fagervall, I., Ross, S. B. (1983) Naunyn-Schmiedeberg's Arch. Pharmacol. 324: 79–87

Ask, A.-L., Högberg, K., Schmidt, L., Kiessling, H., Ross, S. B. (1982) Biochem. Pharmacol. 31: 1401–1406

Cleland, W. W. (1979) Methods in Enzymology Vol. 63A (Ed. Purich, D. L.) pp. 103–138, Academic Press, New York

Fowler, C. J., Oreland, L. (1980) J. Pharm. Pharmacol. 33: 403–406

Fowler, C. J., Tipton, K. F. (1984) Ibid. 36: 111–115

Fowler, C. J., Eriksson, M., Magnusson, O., Thorell, G. (1984) Naunyn-Schmiedeberg's Arch. Pharmacol. 327: 279–284

Fowler, C. J., Wiberg, Å., Oreland, L., Marcusson, J., Winblad, B. (1980) J. Neural Transm. 49: 1–20

Graffner, C., Alvan, G., Lake-Bakaar, D. M., Lindgren, J.-E., Lundström, J., Selander, H. (1983) L'Encephale 9 (Suppl. 1): 20A

Hall, D. W. R., Logan, B. W., Parsons, G. H. (1969) Biochem. Pharmacol. 18: 1447–1454

Lindbom, L.-O., Norrman, S., Hellström, W. (1983) L'Encephale 9 (Suppl. 1): 76A

Markwell, M. A. K., Haas, S. M., Bieber, L. L., Tolbert, N. E. (1978) Analyt. Biochem. 87: 206–210

O'Carroll, A.-M., Fowler, C. J., Phillips, J. P., Tobbia, I., Tipton, K. F. (1983) Naunyn-Schmiedeberg's Arch. Pharmacol. 322: 198–202

Otsuka, S., Kobayashi, Y. (1964) Biochem. Pharmacol. 13: 995–1006

Tipton, K. F., Houslay, M. D., Garrett, N. J. (1973) Nature New Biol. (Lond.) 246: 213–214

Tipton, K. F., Fowler, C. J., McCrodden, J. M., Strolin Benedetti, M. (1983) Biochem. J. 209: 235–242

J. Pharm. Pharmacol. 1985, 37: 354–357
Communicated October 3, 1984

Age-related changes in monoamine oxidase and semicarbazide-sensitive amine oxidase activities of rat aorta

H. Cao Danh*, M. Strolin Benedetti*, P. Dostert*†, A. Mousset, *Centre de Recherche Delalande, 10 rue des Carrières, 92500 Rueil-Malmaison, France*

Aorta MAO-A and SSAO activities were measured on young (3 months) and old (23–26 months) rats. A significant decrease (30–40%) in SSAO activity was found with benzylamine as substrate and the decrease was due to a reduction in V_{max}. No significant changes in MAO-A activity were found in the aorta of old rats. β-PEA is oxidized mainly by SSAO in rat aorta. However, the significance of this is unclear since the physiological role of that enzyme remains unknown.

The heart and aorta of rat and man contain monoamine oxidases (MAO, EC 1.H.3.4) and other amine oxidases (Lyles & Callingham 1975; Lewinsohn et al 1978). Monoamine oxidase exists in two forms (MAO-A and MAO-B) which are differentiated by their specificity to substrates (Tipton et al 1983; Fowler et al 1981) and their sensitivity to inhibitors (Johnston 1968; Knoll & Magyar 1972). One of the other amine oxidases, has been called benzylamine oxidase (BZAO) (Lewinsohn et al 1978, 1980), prefers benzylamine as substrate; it is resistant to inhibition by clorgyline and selegiline ((−)-deprenyl) at concentrations which completely inhibit MAO-A and -B (Lyles & Callingham 1975, 1982a, b) and is sensitive to inhibition by semicarbazide. Therefore, this enzyme has also been called clorgyline-resistant amine oxidase (CRAO) but the preferred name is now semicarbazide-sensitive amine oxidase (SSAO) (Lyles & Callingham 1975; Dial & Clarke 1977; Coquil et al 1973).

Age-related changes in MAO and SSAO activities have been reported in tissues of animals (Lowe et al 1975; Della Corte & Callingham 1977; Fuentes et al 1977; Shih 1979; Strolin Benedetti & Keane 1980; Cao Danh et al 1984, 1985) and man (Robinson et al 1972; Gottfries et al 1975; Oreland & Fowler 1979). The aim of the present work was to compare MAO and SSAO activities in aorta of young and old rats.

† Correspondence.
* Present address: Laboratoires Fournier, Centre de Recherche, 50 rue de Dijon, Daix 21121 Fontaine-lès-Dijon, France

Materials and methods

5-Hydroxytryptamine-[side chain-2-^{14}C]creatinine sulphate (5-HT) and [7-^{14}C] benzylamine hydrochloride (BZ) were obtained from the Radiochemical Centre, Amersham, UK; β-phenethylamine-[ethyl-1-^{14}C] hydrochloride (β-PEA) was obtained from New England Nuclear, Boston, Mass., USA; clorgyline hydrochloride was synthesized in the Department of Organic Chemistry, Centre de Recherche Delalande, France; semicarbazide hydrochloride was obtained from E. Merck, Darmstadt, F.R. Germany. All other reagents were standard laboratory reagents of analytical grade whenever possible.

Male Wistar rats (Iffa Credo, L'Arbresle, France) aged 23–26 months (600–850 g) were compared with matched animals of 3 months (170–180 g). Animals were decapitated. Thoracic aortas were immediately removed, rinsed in saline(0·9% NaCl w/v), frozen in liquid nitrogen, then stored at −20 °C until used.

Aorta MAO and SSAO activity was measured by the micromethod of Clarke et al (1982) with some modifications as described by Guffroy & Strolin Benedetti (1984). In all cases, the final substrate concentrations in the assay tubes were 400 μM for 5-HT, 50 μM for β-PEA and 1 μM or 10 μM for BZ. The initial velocities were measured. MAO and SSAO activity was expressed as nmol (of deaminated substrate) mg^{-1} tissue (or mg protein)$^{-1}$ min^{-1}. Protein concentrations of the homogenates were determined by the method of Lowry et al (1951).

Oxidative deamination of each substrate by MAO-A, -B and/or SSAO in aorta was studied following the decrease of substrate oxidation as a function of in-